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Infection With GB Virus C (GBV-C) in Patients With Chronic Liver Disease or on Maintenance Hemodialysis in Indonesia

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RNA of a non-A to E hepatitis virus identified recently and designated provisionally GB virus C (GBV-C), was sought in patients in Indonesia by reverse-transcription polymerase chain reaction with nested primers deduced from a helicase-like region. GBV-C RNA was detected in 32 (55%) of 58 patients on maintenance hemodialysis at a frequency significantly higher ($P < 0.001$) than that in seven (5%) of 149 patients with chronic liver disease. Co-infection with hepatitis C virus was observed in 26 (81%) of the 32 patients on hemodialysis and in five (71%) of the seven patients with liver disease who were infected with GBV-C. Complete identity was observed in a sequence of 100 base pairs in the helicase-like region for GBV-C cDNA clones from some patients on maintenance hemodialysis. These results indicate that the patients on hemodialysis would be at high risk for GBV-C infection, which would be transmitted by transfusion and patient-to-patient routes.

KEY WORDS: hepatitis viruses, hepatitis B virus, hepatitis C viruses, chronic hepatitis, hemodialysis, epidemiology, blood transfusion

INTRODUCTION

The discovery of hepatitis C virus (HCV) in 1989 [Choo et al., 1989] and its prompt application to the diagnosis of HCV infection by serological and molecular biological techniques [Kuo et al., 1989; Okamoto et al., 1990; Weiner et al., 1990] has been the major breakthrough in the study of non-A, non-B hepatitis. With the advent of these methods, HCV has been established as the major etiological agent for posttransfusion and community-acquired acute and chronic non-A, non-B hepatitis [Houghton et al., 1991]. Further, screening of blood units for antibody to HCV (anti-HCV) was found to be effective for preventing posttransfusion non-A, non-B hepatitis [van der Poel et al., 1989].

It has become increasingly evident, however, that there are patients with acute or chronic hepatitis without evidence for infection with hepatitis A, B, C, D, or E virus, and that there remains a residual risk of posttransfusion hepatitis in the recipients of blood units without hepatitis B surface antigen (HBsAg) or anti-HCV [Alter and Bradley, 1995]. This category of hepatitis will be hereafter referred to as non-A to E hepatitis.

Recently, a putative agent for non-A to E hepatitis has been identified by molecular biological technique from inhabitants and patients of East and West Africa, Canada, and the United States, and designated GB virus C (GBV-C) [Simons et al., 1995a]. It has a genomic organization resembling the *Flaviviridae* family, and is similar in sequence to HCV, albeit with divergence too wide to be classified into HCV genotypes. RNA of GBV-C can be determined by reverse-transcription (RT) polymerase chain reaction (PCR) with primers deduced from its helicase-like region, and has been detected in some patients with fulminant hepatitis of a non-A to E etiology [Simons et al., 1995a; Yoshiba et al., 1995; Zuckerman, 1995]. Very recently, another non-A to E hepatitis has been described with the designation of hepatitis G virus [Linnen et al., 1996]. It is similar in nucleotide and deduced amino acid sequences to GBV-C, and therefore, considered to be the same virus of probably distinct genotypes.

We have reported different profiles of HCV infection in patients with chronic liver disease and those treated by maintenance hemodialysis in Indonesia [Hadiwandowo et al., 1994]. The same cohort of patients were tested for GBV-C RNA.

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TABLE I. RNA of GB Virus C (GBV-C), and Markers of Other Hepatitis Viruses Infection in Patients With Chronic Liver Disease or on Maintenance Hemodialysis in Indonesia

Disease[a]	N	GBV-C RNA[b]	HBsAg	Anti-HCV	HCV RNA[c]
CH	24	2 (8%)	10 (42%)	8 (33%)	6/8 (75%)
LC	86	4 (5%)	24 (28%)	39 (45%)	29/39 (74%)
HCC	39	1 (3%)	6 (15%)	17 (44%)	13/17 (76%)
Total	149	7 (5%)	40 (27%)	64 (43%)	48/64 (75%)
CRF	58	32 (55%)	4 (7%)	46 (79%)	44/46 (96%)

[a]CH, chronic hepatitis; LC, liver cirrhosis; HCC, primary hepatocellular carcinoma; CRF, chronic renal failure.
[b]Determined by RT-PCR with nested primers deduced from a helicase-like region of GBV-C (see Methods).
[c]Tested only in sera positive for anti-HCV.

MATERIALS AND METHODS

Patients

Sera were obtained from consecutive patients with chronic liver disease who were admitted or visiting two hospitals in Yogyakarta, Indonesia, during 9 months from April to November, 1992, and had been stored at −20°C [Hadiwandowo et al., 1994]. There were 149 patients (age 51 ± 13 years; male 108, female 41) including 24 with chronic hepatitis, 86 with liver cirrhosis, and 39 with primary hepatocellular carcinoma. Diagnosis was based on transaminases, gammaglobulins, alfa-fetoprotein, and ultrasonography; some patients had liver biopsy for histological diagnosis. Essentially all of them were Moslems and refrained from alcohol intake. Tests for non-viral causes of liver disease, such as autoimmune hepatitis and metabolic disorders, were not carried out on all of them. Also studied were 58 patients (age 49 ± 13 years; male 42, female 16) who were treated by maintenance hemodialysis in Sardjito Hospital [Hadiwandowo et al., 1994]. The study was approved by the Ethics Committee of the hospital, and all the patients gave an informed consent.

Determination of GBV-C RNA by RT-PCR

This was undertaken by RT-PCR with nested primers, deduced from a helicase-like sequence in the non-structural (NS) 3 region of the GBV-C genome, after the method described previously [Egawa et al., 1996]. Briefly, nucleic acids were extracted from 100 μl of serum, reverse-transcribed to cDNA and subjected to amplification with primers #G8 (sense)/#G9 (antisense) followed by that with #G10 and #G11. The amplification of the target sequence was confirmed by the size of RT-PCR products, i.e., 159 and 83 base pairs (bp), respectively.

Nucleotide Sequences of GBV-C Isolates

Products of PCR with primers #G8 and #G9 were amplified by a heminested PCR with primers #G8 and #G11 [Yoshiba et al., 1995], and cloned into M13 phage vectors. A sequence of 100 bp in the helicase-like region of GBV-C was determined on three clones each from a serum, and the consensus sequence was adopted.

Serological Tests

HBsAg was determined by a sandwich immunoassay with horse polyclonal and murine monoclonal antibodies as described previously [Hadiwandowo et al., 1994]. Antibody to HCV (anti-HCV) was determined by enzyme immunoassay of the second generation (EIA-II, Ortho Diagnostic Systems, Tokyo, Japan) with absorbance at 492 nm (A_{492}) values >0.63 considered reactive. Serum samples reactive by EIA-II were tested for HCV RNA by RT-PCR with primers deduced from the well-conserved 5′-noncoding region of the HCV genome [Okamoto et al., 1994].

Statistical Analysis

Frequencies between groups were evaluated using the χ^2 test and Fisher's exact test.

RESULTS

Table I compares markers of GBV-C, hepatitis B virus, and HCV infections in patients with chronic liver disease and those treated by maintenance hemodialysis in Indonesia. GBV-C RNA was detected in 32 (55%) of 58 patients on maintenance hemodialysis at a frequency significantly higher than that in seven (5%) of 149 patients with chronic liver disease ($P < 0.001$). Anti-HCV was detected in 46 (79%) of 58 patients on maintenance hemodialysis and in 64 (43%) of 149 patients with chronic liver disease. HCV RNA was tested only in patients with anti-HCV, and was detected in 44 (96%) of 46 dialysis patients with the antibody, signficantly more frequently ($P < 0.01$) than in 48 (75%) of 64 patients with liver disease with the antibody.

There were no differences in the frequency of the other hepatitis virus markers in patients with different liver disease, except that the frequency of HBsAg was a little lower in the patients with hepatocellular carcinoma than in those with the other liver disease (6/39 or 15% vs. 34/110 or 31%).

Demographic, clinical, and virological features were compared between the patients with chronic liver disease who were positive for GBV-C RNA in the serum and those who were not (Table II). Anti-HCV was detected in six (86%) of the seven patients with GBV-C RNA at a

TABLE II. Comparison of Patients With Chronic Liver Disease With and Without GBV-C RNA in Serum

Features	Detection of GBV-C RNA Positive (N = 7)	Detection of GBV-C RNA Negative (N = 142)	Differences
Male	4 (57%)	104 (73%)	N.S.[a]
Age (years)	54 ± 11	50 ± 13	N.S.
Disease			N.S.
CH	2 (28%)	22 (15%)	N.S.
LC	4 (57%)	82 (58%)	N.S.
HCC	1 (14%)	38 (27%)	N.S.
HBsAg	1 (14%)	39 (27%)	N.S.
Anti-HCV	6 (86%)	58 (41%)	$P < 0.05$
HCV RNA	5 (71%)	43 (30%)	N.S.

[a]N.S., not significant.

TABLE III. Comparison of Patients on Maintenance Hemodialysis With and Without GBV-C RNA in Serum

Features	Detection of GBV-C RNA Positive (N = 32)	Detection of GBV-C RNA Negative (N = 26)	Differences
Male	22 (69%)	20 (77%)	N.S.[a]
Age (years)	49 ± 14	48 ± 13	N.S.
HBsAg	2 (6%)	2 (8%)	N.S.
Anti-HCV	26 (81%)	20 (77%)	N.S.
HCV RNA	26 (81%)	18 (69%)	N.S.

[a]N.S., not significant.

frequency significantly higher ($P < 0.05$) than that in 58 (41%) of the 142 patients without GBV-C RNA. There were no appreciable differences in the other features in comparison, although HCV RNA was detected a little more frequently in the patients with GBV-C RNA than in those without.

Table III compares various features between the patients treated by maintenance hemodialysis who possessed GBV-C RNA in serum and those who did not. The detection of HCV RNA was more frequent in patients with GBV-C RNA than in those without (26/32 or 81% vs. 18/26 or 69%) but the difference was not significant.

Consensus nucleotide sequences of 100 bp in the helicase-like region of three each GBV-C cDNA clones from the seven patients with chronic liver disease (Cases 1–7) and 13 patients (Cases 8–20) selected randomly from the 32 patients on maintenance hemodialysis are shown in Fig. 1, in comparison with previously reported sequences. The sequences from the seven patients with chronic hepatitis differed 3–21%, and none of these were identical. By contrast, the sequences from 13 dialysis patients differed by 0–19%. Remarkably, sequences from Cases 8–11 and those from Cases 12–14 were identical or differed by only 1%, and those from Cases 15–17 were the same.

Overall, Indonesian GBV-C strains represented by the 20 patients were similar to the American and African strains [Simons et al., 1995] by 75–89% and to the Japanese isolates from patients with fulminant hepatitis [Yoshiba et al., 1995] by 78–91%.

DISCUSSION

The recognition of GBV-C dates back some 30 years ago to a surgeon (G.B.) with a retrospective diagnosis of non-A to E hepatitis [Deinhardt et al., 1967]. The preacute serum was propagated through 11 passages in tamarins and two viral agents were isolated with names of GB virus A and B (GBV-A and GBV-B) [Simons et al., 1995b]. One of these (GBV-B) induced liver injury in tamarins [Schlauder et al., 1995]. By enzyme immunoassay with antigen probes of these two animal viruses, a possible human counterpart was detected in symptom-free individuals or patients with acute or chronic hepatitis from different parts of the world, and named GBV-C [Simons et al., 1995a]. GBV-C resembles GBV-A in sequence more closely than GBV-B [Simons et al., 1995a].

Little is known about the epidemiology, mode of transmission, and disease-inducing activity of GBV-C. Antibodies to GBV-C have been detected in 7% of multitranfused patients and in 1.8% of drug abusers [Zuckerman, 1995], pointing to its blood-borne nature. They were found in 15% of blood donors in West Africa and 2% of those in the United States, thereby indicating marked regional differences in the epidemiology of GBV-C [Zuckerman, 1995].

The diagnosis of GBV-C infection has become possible by RT-PCR with nested primers deduced from a helicase-like region in the NS3 region [Simons et al., 1995a; Yoshiba et al., 1995; Egawa et al., 1996]. With the advent of this technique, GBV-C RNA has been detected in three patients with fulminant hepatitis without evidence for the other hepatitis virus infections [Yoshiba et al., 1995], thereby indicating a role of GBV-C in inducing this disease. Since by far the most patients with non-A, non-B fulminant hepatitis are not infected with HCV [Fagan and Harrison, 1994; Mutimer et al., 1995; Wright, 1993], GBV-C would be expected to account for some patients with this extremely severe form of hepatitis. In contrast with the implication of GBV-C in fulminant hepatitis, however, non-A to E hepatitis occurring after transfusion or by community-acquired infection is reported to run mild clinical course mostly [Alter and Bradley, 1995].

RNA of GBV-C was detected in 32 (55%) of 58 patients treated by maintenance hemodialysis in Indonesia. Infection with HCV was common, involving 46 (79%) patients. Infection with HCV of genotype I/1a was detected in 39 (89%) of the 44 dialysis patients with HCV RNA, at a frequency much higher than in none of the 48 patients with chronic liver disease with HCV RNA in Indonesia [Hadiwandowo et al., 1994]. Hence, GBV-C would have spread among dialysis patients by routes of transmission similar to those of HCV such as transfusion and shared devices for their treatment.

Comparison of 100-bp sequence of the helicase-like region of GBV-C clones from patients on maintenance hemodialysis indicated patient-to-patient transmission. Sequences from three patients were identical and those from other three and four patients, respectively, were the same or differed by only 1%. This contrasted with

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                         *         *         *         *         *         *         *         *         *         *
 1) GBV-C      CGAGCGTATGAGGACTGGTCGCCACCTTGTATTCTGCCATTCCAAGGCGGAGTGCGAGAGATTGGCCGGCCAGTTCTCCGCGCGGGGGGTTAATGCCATC
 2) GBV-C.1    ------------------------------------------------------------------T---------------------------------
 3) GBV-C.2    ------G---C----C--G--------C--G-----------A---------------C-G-----T-----------TT----------G--------T
 4) GBV-C.3    G---A-G---C----C--CA-G-----C--------------A---------------C-GC-T--T-----------A--C--------A--------T
 5) GBV-C.4    G-----G--------C---A-G-----G-----------C--A-----------T-----GC----------A------T-A--------------TG-T
 6) GBV-C.5    ---A--G---C----C--AA-G-----C--G-----------A---------------C-GC-C--T--T-----T--T---A-------A--C-----T
 7) GBV-C.6    G-----G---CA---C---A-A--T-----G---C----C--G---------------C-GC-T---------------T-TA-------C--C-----T
 8) GBV-C.7    ---A--G---C-A--C--AG-G-----C--G-----T---------------------C-GC-T--T-----------T---A-------G--------T
 9) FH #1      T-----G---C-A--C--A--G-----C--G-----------------T---------C-CC-C-----T--------TT-TA----A--C-----T---
10) FH #2      G-----G---C-A--C--CA-G--------G--T--T-----A-----A---------C-CC----T--------T---T-TA-------C--C------
11) FH #3      G-----G---C-A--C--CA-G---T-G--------------A---------------C-C-----------A------T----------A--C-----A
12) Case  1    ------G---C-A--C--CA-G-----G-----------C--------T---------C-C-----G--------T-----AA-------C---------
13) Case  2    G-----A------------A-G-----C--------------A-----------T---C-CC----G-----A------T-C--------C--------T
14) Case  3    T-----G---C----C--CA-G---T-A--G--------C--T---------------C-C-----G--------T--T--TA-A--------C-----T
15) Case  4    T-----G---C-------GA-G-----C--G-----------A-----------T---C-CC-A--G-----A-----AAGCA----T--C--C-----T
16) Case  5    G---------C----A--C--A---T-G-------------------------------C-T-----T-----A--T---T-CA----C--C--C------
17) Case  6    G-----G---C----C---A-G--T--C--G--------C--G---------------C-C-----G-----------TT-C-----------------T
18) Case  7    T-----G---C----C--CA-G---T-A-----------C------------------C-C-----G--------T--T--TA----------C-----T
19) Case  8    G-----G---C----G--CA-G-----C--G---------------------------C-C--------A--A-----GT-C--A-----A-----T--T
20) Case  9    G-----G---C----G--CA-G-----C--G---------------------------C-C--------A--A-----GT-C--A-----A-----T--T
21) Case 10    G-----G---C----G--CA-G-----C--G---------------------------C-C--------A--A-----GT-C--A-----A-----T--T
22) Case 11    G-----G---C----G--CA-G-----C--G---------------------------C-C--------A--A-----GT-C--A-----G-----T--T
23) Case 12    G-----G---C----C--GA-G--T--C--G--------C--A---------------C-CC----G-----A-----G--CA----T--C-----T---
24) Case 13    G-----G---C----C--GA-G--T--C--G--------C--A---------------C-CC----G-----A-----G--CA----T--C-----T---
25) Case 14    G-----G---C----C--GA-G--T--C--G--------C--A---------G-----C-CC----G-----A-----G--CA----T--C-----T---
26) Case 15    G---A-G---C-------CA-G-----C--------------G--------------AC-CC----G-----A-----TT-C--------C--------T
27) Case 16    G---A-G---C-------CA-G-----C--------------G--------------AC-CC----G-----A-----TT-C--------C--------T
28) Case 17    G---A-G---C-------CA-G-----C--------------G--------------AC-CC----G-----A-----TT-C--------C--------T
29) Case 18    G-----G---C----G--C--G--T--C--G--------C--------A---------C-CC-------A--A-----GT-C--------A-----T---
30) Case 19    T-----G---C----C--CA-G---T-G------------------------------C-C-----G-----A--T--T--TA-------C--C-----T
31) Case 20    G-----G---C-C--G---A-G---T-A------------------------------C-C--------G--A-----GT-C-----------C-----A
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Fig. 1. Nucleotide sequences of 100 base pairs in the helicase-like region of GB virus C (GBV-C). The consensus sequences of 3 GBV-C cDNA clones each from seven patients with chronic liver disease (Cases 1–7) and 13 patients on maintenance hemodialysis (Cases 8–20) in Indonesia are shown. Reported sequence of GBV-C isolates from America and Africa (GBV-C and GBVC.1–7 [Simons et al., 1995]) and those from Japan (FH#1–3 [Yoshiba et al., 1995]) are indicated above.

sequences from patients with chronic liver disease which differed by 3–21%. Overall, sequences of 20 Indonesian GBV-C isolates differed from those of American and African isolates by 11–25%, and from Japanese isolates by 9–22%. Hence, there would be distinct GBV-C strains which have different geographic distributions, as is the case with HCV genotypes [Houghton et al., 1991].

The present results attest to a mode of GBV-C transmission similar to that of HCV transmission. Hence, individuals at high risk for HCV infection, such as patients with hemophilia [Brettler et al., 1990] and drug abusers [Alter et al., 1990] are also expected to be at increased risk for GBV-C infection. GBV-C RNA was detected more often in patients with chronic hepatitis C who were drug abusers than in those who were not [Aikawa et al., 1996]. Inasmuch as GBV-C infection is incriminated in fulminant hepatitis, the screening of blood units for GBV-C may have some impact in preventing posttransfusion non-A to E fulminant hepatitis. The development of serological assays for detecting GBV-C infection is awaited for screening blood units and to delineate the mode of its transmission and sequelae.

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